

REMARKS

In view of the preceding amendments and the comments which follow, and pursuant to 37 CFR §1.116, amendment and reconsideration of the Official Action of September 6, 2002 is respectfully requested by Applicant.

Amendments to specification

The title has been amended pursuant to the Examiner's requirement.

"Clean" and "marked up" versions of page 1 of the specification are attached hereto.

Amendments to claims

Claims 46-53 have been cancelled without prejudice as being drawn to a non-elected invention.

Claims 18, 26, 27, 37 and 38 have been amended to characterize the degree of stability of the coenzyme solution. Support for the added language is found in the specification on page 6, lines 18-22 and in Tables 1 and 2.

"Clean" and "marked up" versions of currently pending claims 18-45 are attached hereto.

Rejection under 35 USC §103 (a)

The Examiner has maintained the rejection of claims 18-45 under 35 USC §103 (a) as being unpatentable over U.S. Patent No. 5,424,204 issued to Aoyama *et al.* (hereinafter "Aoyama"). It is the Examiner's position that, as claimed, the invention is obvious over Aoyama because no degree of stability is claimed that would render Aoyama distinct from the claims. Further, the compositions of Aoyama are described as

stable and therefor it would be presumed all the components of the compositions would be stable including the NADH.

In response, Applicant has amended claims 18, 26, 27, 37 and 38 to characterize the degree of stability of the coenzyme solution as remaining qualitatively unchanged following treatment at 42 °C for 5 days (see page 6, lines 16-22, and Tables 1 and 2 of the specification). The remaining claims are dependent from claims 18, 26, 27, 37 and 38 and are thereby similarly limited. This distinguishes the claimed invention from that of Aoyama, who describes stabilization of the enzyme glucose-6-phosphate dehydrogenase in a liquid clinical assay reagent which is used for quantitatively determining the amount of a glucose-related substance (see abstract; column 1, lines 51-56; column 5, lines 38-40; Example 1 and Example 2). In Table 5, Aoyama shows stability of some solutions with added hydroxylamine compounds following storage at 10 °C for 3 months; however, following storage for 12 months, a significant loss in stability was observed in all solutions. No solution of Aoyama was demonstrated to remain stable for 12 months at 10 °C, while Applicant teaches a corresponding stability of 15 months at 2 to 8 °C (page 6, lines 16-18).

As now amended, Applicant claims a degree of stability that renders Aoyama distinct from the claims. Thus, Applicant argues that the Examiner has failed to make a *prima facie* case of obviousness, and reconsideration of the rejection of claims 18-45 is respectfully requested.

Applicants submit that their application is now in condition for allowance, and favorable reconsideration of their application in light of the above amendments and remarks is respectfully requested. Allowance of claims 18-45 at an early date is earnestly solicited.

The Examiner is hereby authorized to charge any fees associated with this Amendment to Deposit Account No. 02-2958. A duplicate copy of this sheet is enclosed.

Respectfully submitted,

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~~Stabilized coenzyme solutions and their use for determining
dehydrogenases or substrates thereof~~

STABILIZED COENZYME SOLUTIONS

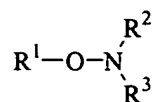
The invention concerns stabilized aqueous solutions of a coenzyme for hydrogen-transferring enzymes and their use for determining a corresponding analyte (substrate) in a reduced form or for determining the enzyme activity of a corresponding dehydrogenase. The stabilized solution contains an organic compound or appropriate salts having a pKa value between 1.5 and 6.0 and/or a hydroxylamine derivative.

The determination of enzyme activities (or substrate concentrations), especially in blood serum or plasma, plays an important role in clinical chemical diagnostics. Test procedures are often used for this which are based on the reduction of nicotinamide adenine dinucleotide ("NAD") or nicotinamide adenine dinucleotide phosphate ("NADP") and photometric detection of the resulting change of the absorption behaviour in the ultraviolet wavelength range ($\lambda = 334, 340$ or 365 nm). When suitable test conditions have been selected, this change is linearly proportional to the enzyme activity (or substrate concentration) to be determined.

Nowadays the methods described in Eur. J. Clin. Chem. Clin. Biochem. 31, 897 (1994) and Eur. J. Clin. Chem. Clin. Biochem. 32, 639 (1994) are generally recommended for determining the enzyme activity of for example lactate dehydrogenase (LDH, E.C.1.1.1.27). The test

What is claimed is:

18. An aqueous solution comprising a hydrogen-accepting coenzyme selected from the group consisting of NAD, NADP and derivatives thereof and one or more compounds selected from the group consisting of organic compounds or salts thereof having a pKa value between 1.5 and 6.0 and nitrogen compounds of the formula



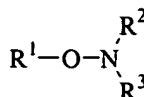
in which R¹, R² and R³ are the same or different and denote hydrogen or a saturated or unsaturated alkyl or aryl group, the solution characterized by remaining qualitatively unchanged following treatment at 42 °C for 5 days.

19. The solution of claim 18 wherein the organic compound is citric acid or a citrate salt.
20. The solution of claim 19 wherein the concentration of the citric acid or citrate salt is about 5 to 200 mM.
21. The solution of claim 18 wherein the pH is between 1.0 and 7.0.
22. The solution of claim 18 wherein the nitrogen compound is a hydroxylamine derivative or salt thereof.
23. The solution of claim 22 wherein the concentration of the hydroxylamine derivative or salt is between about 2 and 300 mM.
24. The solution of claim 18 further comprising a boric acid derivative.

25. The solution of claim 24 wherein the concentration of the boric acid derivative is about 50 to 200 mM.
26. A method for determining the concentration of a hydrogen-transferring substrate in a sample comprising:
- (a) forming a reaction mixture by combining the sample with a hydrogen-transferring enzyme for the substrate, a hydrogen-accepting coenzyme selected from the group consisting of NAD, NADP and derivatives thereof, and one or more compounds selected from the group consisting of organic compounds or salts thereof having a pKa value between 1.5 and 6.0 and nitrogen compounds of the formula
$$\begin{array}{c} \text{R}^2 \\ | \\ \text{R}^1-\text{O}-\text{N} \\ | \\ \text{R}^3 \end{array}$$
in which R¹, R² and R³ are the same or different and denote hydrogen or a saturated or unsaturated alkyl or aryl group, the enzyme, coenzyme and one or more compounds characterized by remaining qualitatively unchanged following treatment at 42 °C for 5 days, and
 - (b) detecting the change in absorbance of the coenzyme as a measure of the concentration of the substrate present in the sample.
27. A method for determining the activity of a hydrogen-transferring enzyme in a sample comprising:
- (a) forming a reaction mixture by combining the sample with a hydrogen-transferring substrate for the enzyme, a hydrogen-accepting coenzyme selected from the group consisting of NAD, NADP and derivatives

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thereof, and one or more compounds selected from the group consisting of organic compounds or salts thereof having a pKa value between 1.5 and 6.0 and nitrogen compounds of the formula

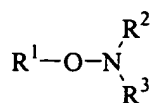


in which R¹, R² and R³ are the same or different and denote hydrogen or a saturated or unsaturated alkyl or aryl group, the enzyme, coenzyme and one or more compounds characterized by remaining qualitatively unchanged following treatment at 42 °C for 5 days, and

- (b) detecting the change in absorbance of the coenzyme as a measure of the activity of the enzyme present in the sample.
28. The method of claim 26 wherein the hydrogen-transferring substrate is selected from the group consisting of lactate, glutamate, ammonia, alcohol, glyceraldehyde-3-phosphate and glucose.
29. The method of claim 27 wherein the enzyme is selected from the group consisting of dehydrogenases of lactate, glutamate, alcohol, glycerol-3-phosphate and glucose.
30. The method of claim 26 or 27 wherein the organic compound is citric acid or a citrate salt.
31. The method of claim 30 wherein the concentration of the citric acid or citrate salt is about 5 to 200 mM.

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32. The method of claim 26 or 27 wherein the pH of the reaction mixture is between about 8.5 and 10.0.
33. The method of claim 26 or 27 wherein the nitrogen compound is a hydroxylamine derivative or salt thereof.
34. The method of claim 33 wherein the concentration of the hydroxylamine derivative or salt is between about 2 and 300 mM.
35. The method of claim 26 or 27 wherein the reaction mixture further comprises a boric acid derivative.
36. The method of claim 35 wherein the concentration of the boric acid derivative is about 50 to 200 mM.
37. A kit for determining the concentration of a hydrogen-transferring substrate in a sample comprising:
- (a) a first reagent comprising a hydrogen-transferring enzyme for the substrate in a buffer having a pH between about 8.5 and 10.0 and
 - (b) a second reagent comprising a hydrogen-accepting coenzyme selected from the group consisting of NAD, NADP and derivatives thereof and one or more compounds selected from the group consisting of organic compounds or salts thereof having a pKa value between 1.5 and 6.0 and nitrogen compounds of the formula

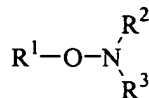


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in which R^1 , R^2 and R^3 are the same or different and denote hydrogen or a saturated or unsaturated alkyl or aryl group, the second reagent characterized by remaining qualitatively unchanged following treatment at 42 °C for 5 days.

38. A kit for determining the activity of a hydrogen-transferring enzyme in a sample comprising:

- (a) a first reagent comprising a hydrogen-transferring substrate for the enzyme and a buffer having a pH between about 8.5 and 10.0 and
- (b) a second reagent comprising a hydrogen-accepting coenzyme selected from the group consisting of NAD, NADP and derivatives thereof and one or more compounds selected from the group consisting of organic compounds or salts thereof having a pKa value between 1.5 and 6.0 and nitrogen compounds of the formula



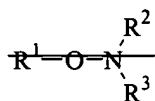
in which R^1 , R^2 and R^3 are the same or different and denote hydrogen or a saturated or unsaturated alkyl or aryl group, the second reagent characterized by remaining qualitatively unchanged following treatment at 42 °C for 5 days.

- 39. The kit of claim 37 or 38 wherein the organic compound is citric acid or a citrate salt.
- 40. The kit of claim 39 wherein the concentration of the citric acid or citrate salt is about 5 to 200 mM.

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41. The kit of claim 37 or 38 wherein the second reagent has a pH between about 1.0 and 7.0.
42. The kit of claim 37 or 38 wherein the nitrogen compound is a hydroxylamine derivative or salt thereof.
43. The kit of claim 42 wherein the concentration of the hydroxylamine derivative or salt is about 2 to 300 mM.
44. The kit of claim 37 or 38 wherein the first reagent further comprises a boric acid derivative.
45. The kit of claim 44 wherein the concentration of the boric acid derivative is about 50 to 200 mM.

46. ~~A method for stabilizing an aqueous solution comprising a hydrogen-accepting coenzyme selected from the group consisting of NAD, NADP and derivatives thereof, the method comprising adding to the solution one or more compounds selected from the group consisting of organic compounds or salts thereof having a pKa value between 1.5 and 6.0 and nitrogen compounds of the formula~~



- ~~in which R¹, R² and R³ are the same or different and denote hydrogen or a saturated or unsaturated alkyl or aryl group.~~
47. ~~The method of claim 46 wherein the organic compound is citric acid or a citrate salt.~~

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- ~~48. The method of claim 47 wherein the concentration of the citric acid or citrate salt is about 5 to 200 mM.~~
- ~~49. The method of claim 46 wherein the pH is between 1.0 and 7.0.~~
- ~~50. The method of claim 46 wherein the nitrogen compound is a hydroxylamine derivative or salt thereof.~~
- ~~51. The method of claim 50 wherein the concentration of the hydroxylamine derivative or salt is between about 2 and 300 mM.~~
- ~~52. The method of claim 46 further comprising a boric acid derivative.~~
- ~~53. The method of claim 52 wherein the concentration of the boric acid derivative is about 50 to 200 mM.~~